Clinical Utility of Serum Cystatin C in Predicting Diabetic Nephropathy Among Patients with Diabetes Mellitus: a Meta-Analysis

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Key Words
Diabetic nephropathy • Serum cystatin C • Glomerular filtration rate • Meta-analysis

Abstract
Background/Aims: Clinically, there is lack of predictors for diabetic nephropathy (DN) in diabetes mellitus (DM) without microalbuminuria, macroalbuminuria or retinopathy.
Methods: PubMed, Chinese Biomedical Database, Cochrane Library, EMBASE and Elsevier Database were searched from inception to August 13, 2016. Studies involving patients with DM and containing data on cystatin C measurements and the measured glomerular filtration rate (mGFR) were included. Pooled sensitivity, specificity, positive predictive value, negative predictive value and other diagnostic indices were evaluated using a random effect model.
Results: The meta-analysis enrolled 9 studies with 1417 patients. The pooled sensitivity and specificity of serum cystatin C for predicting DN were 0.88 (95\% CI 0.85 - 0.91) and 0.85 (95\% CI 0.82 - 0.87), respectively. The pooled positive and negative predictive values of serum cystatin C for predicting DN were 7.04 (95\% CI 4.33 - 11.43) and 0.13 (95\% CI 0.09 - 0.20), respectively. The area under the summary receiver operating characteristic (SROC) curve was 0.9549, and the diagnostic odds ratio was 66.80 (95\% CI 27.92 - 159.86). Conclusion: Serum cystatin C is an early predictor of DN among patients with DM.

B. Zhou and H. Zou contributed equally to this work and therefore share first authorship.
Introduction

Diabetic nephropathy (DN) is one of the major chronic microvascular complications in diabetes mellitus (DM) and a leading cause of end-stage renal disease (ESRD), accounting for nearly half of all incident cases of ESRD in the developed world [1, 2]. In 2014, the American Diabetes Association (ADA) and the National Kidney Foundation (NKF) reached an agreement in which DN was referred to as the chronic kidney disease caused by DM, with a persistent estimated glomerular filtration rate (eGFR) of < 60 ml per min per 1.73 m$^2$ or a urinary albumin/creatinine ratio (ACR) of > 30 mg/g for more than 3 months [3]. The Joint Committee on Diabetic Nephropathy recommended the combination of the 24-hour albuminuria excretion rate (AER) and glomerular filtration rate (GFR) as the new classification of DN [4], and AER and spot ACR are considered the gold standard in diagnostic tests for DN [5]. Microscopically, DN is characterized by diffuse or nodular glomerulosclerosis, afferent and efferent hyaline arteriolosclerosis, and tubulointerstitial fibrosis and atrophy [6].

The progression of DN is accompanied by a decrease in GFR among patients with DM. GFR and albuminuria have long been considered twin manifestations of DN [7]. However, research has shown that normoalbuminuric patients with DM may have significant renal damage [8, 9]. The Diabetes Control and Complications Trial (DCCT)/ Epidemiology of Diabetes Interventions and Complications (EDIC) study demonstrated that 24% of the examined DM patients with baseline eGFR level > 60 ml per min per 1.73 m$^2$ progressed to DN within one year; however, they did not experience microalbuminuria or macroalbuminuria [10]. Therefore, GFR is superior to AER or ACR in the early diagnosis of DN. Recently, serum cystatin C has been considered a new biomarker for the diagnosis of kidney damage. Several studies have shown that serum cystatin C is a better marker of decreased GFR than serum creatinine is [11-15].

In this study, we aimed to evaluate the GFR via serum cystatin C for the early detection of DN among DM patients. We conducted the meta-analysis to systematically analyze the relevant studies for determining the predictive value of serum cystatin C for the early detection of DN.

Materials and Methods

Data Sources and Search Strategy

The meta-analysis was performed according to the Observational Studies in Epidemiology (MOOSE) guidelines [16]. We performed a computerized search of PubMed (1966 to August 2016), Chinese Biomedical Database (1978 to August 2016), Cochrane Library (1900 to August 2016), EMBASE (1988 to August 2016) and Elsevier (2006 to August 2016), to identify potentially relevant articles. The medical subject headings (MeSH) were 'serum cystatin c' and 'diabetic nephropathy' AND 'glomerular filtration rate' AND (sensitivity OR specificity OR accuracy). The list of articles was reviewed by two authors (B-Q.Z. and H-H.Z.), who read the full text of all studies that were identified and determined their suitability for inclusion in the review, based on whether they met the pre-specified inclusion criteria. Reference lists of the identified articles were also reviewed manually to identify additional articles.

Study Selection

Two reviewers independently reviewed potentially relevant articles for eligibility and inclusion. Disagreement was resolved via discussion. Studies involving serum cystatin C predicting DN and giving the measured glomerular filtration rate (mGFR) for patients with DM were enrolled in this meta-analysis. There were no language or publishing date restrictions. The age of the subjects was not limited. Studies that involved patients with severe infection, diabetic ketoacidosis, heart failure, primary kidney disease, malignancy and drug-related renal damage were excluded. Studies that did not provide mGFR (sensitivity OR specificity OR accuracy) for patients were also excluded.
Data Extraction and Quality Assessment

Two independent researchers participated in the data extraction of the included studies. Disagreements about extracted data were resolved through discussion. The following variables were documented or recalculated: type of DM, measurement method of serum cystatin C, cut off value for serum cystatin C, specificity, sensitivity, and the area under the receiver operating characteristic curve (AUROC). Quality assessment, in each included study, was performed according to the quality assessment of studies diagnostic (QUADAS) document [17].

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**Fig. 1.** Flow chart of study selection. DN, diabetic nephropathy; GFR, glomerular filtration rate; eGFR, measured glomerular filtration rate; mGFR, estimated glomerular filtration rate.
Statistical analysis

Statistical analysis was performed using the Meta-Disc 1.4 software. I² was used to examine the heterogeneity, with I² > 50% considered significant. If I² > 50%, the random effects model was used for meta-analysis; otherwise, the random effects model was selected. The diagnostic test accuracy of serum cystatin C for detecting DN was expressed as the area under the summary receiver operating characteristic (SROC) curve.

Results

Search Results and Study Characteristics

The crude search initially yielded 101 articles. Of these, 78 were excluded after reading the abstracts and titles because they were review articles, animal studies, or irrelevant to the present analysis. We also read the potential relevant articles that were found by browsing the reference lists of the related articles and reviews and identified 5 additional articles eligible for our meta-analysis. After a careful and thorough screening process, we finally included 9 studies with a total of 1417 patients in the meta-analysis (figure 1). The characteristics of the included studies are listed in Table 1. All studies were published in English and represent international experiences from seven countries. Most studies were conducted in patients with type 2 DM. The detection methods of serum cystatin C included particle-enhanced turbidimetric immunoassay (PETIA) and particle enhanced nephelometric immunoassay (PENIA). GFR was laboratory obtained by testing the plasma clearance of 51Cr-EDTA [18-23], creatinine [24, 25] or 99mTc-DTPA [26]. Thresholds of serum cystatin C are listed in table 1.

Quality Assessment

QUADAS entries were adopted to improve the evaluation quality in the literature; each study is described with a "yes", "no", or "not clear" evaluation. The quality of the evaluation studies is shown in table 2. Four studies did not explain whether the target condition changed between the reference standard and index test [18-20, 23, 24].

Meta-analysis

Heterogeneity analysis. The heterogeneity of sensitivity was (I² = 54.1%, P = 0.0258). The heterogeneity of specificity was (I² = 86.3%, P = 0.0000). The heterogeneity of the positive likelihood ratio and negative likelihood ratio was (I² = 81.2%, P = 0.0000) and (I² = 50.2%, P = 0.0414), respectively. The heterogeneity of the diagnostic odds ratio (DOR) was (I² = 71.6%, P = 0.0005).

Combined analysis. The analyses demonstrated that there was modest heterogeneity among the 7 studies. Our meta-analysis combined the effects using the random effects model.

<table>
<thead>
<tr>
<th>Study [first author]</th>
<th>Country of origin</th>
<th>Sample size</th>
<th>Type of DN</th>
<th>Cystatin C method</th>
<th>TP</th>
<th>FP</th>
<th>FN</th>
<th>TN</th>
<th>The method of GFR</th>
<th>Threshold of Serum cystatin C (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Christesson AG²⁸</td>
<td>Sweden</td>
<td>123</td>
<td>Type 1 and 2</td>
<td>PETIA</td>
<td>14</td>
<td>3</td>
<td>1</td>
<td>105</td>
<td>51Cr-EDTA</td>
<td>Not given</td>
</tr>
<tr>
<td>Beauvieux MC²⁹</td>
<td>France</td>
<td>124</td>
<td>Type 1 and 2</td>
<td>PENIA</td>
<td>70</td>
<td>6</td>
<td>6</td>
<td>42</td>
<td>51Cr-EDTA</td>
<td>0.95</td>
</tr>
<tr>
<td>Rigalieu V²⁰</td>
<td>France</td>
<td>124</td>
<td>Type 1 and 2</td>
<td>PENIA</td>
<td>70</td>
<td>9</td>
<td>6</td>
<td>39</td>
<td>51Cr-EDTA</td>
<td>1.10</td>
</tr>
<tr>
<td>Iliadis F²¹</td>
<td>Greece</td>
<td>448</td>
<td>Type 2</td>
<td>PETIA</td>
<td>118</td>
<td>64</td>
<td>27</td>
<td>242</td>
<td>51Cr-EDTA</td>
<td>Not given</td>
</tr>
<tr>
<td>Bece S²²</td>
<td>Slovenia</td>
<td>113</td>
<td>Type 2</td>
<td>PENIA</td>
<td>73</td>
<td>0</td>
<td>13</td>
<td>27</td>
<td>51Cr-EDTA</td>
<td>Not given</td>
</tr>
<tr>
<td>Odlozova C²³</td>
<td>France</td>
<td>49</td>
<td>Not given</td>
<td>PENIA</td>
<td>9</td>
<td>4</td>
<td>1</td>
<td>35</td>
<td>51Cr-EDTA</td>
<td>Not given</td>
</tr>
<tr>
<td>Chae HW²⁴</td>
<td>Korea</td>
<td>113</td>
<td>Type 1 and 2</td>
<td>PENIA</td>
<td>17</td>
<td>31</td>
<td>3</td>
<td>62</td>
<td>creatinine</td>
<td>Not given</td>
</tr>
<tr>
<td>Bicik Z²⁵</td>
<td>Turkish</td>
<td>72</td>
<td>Type 2</td>
<td>PETIA</td>
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<td>4</td>
<td>3</td>
<td>36</td>
<td>creatinine</td>
<td>1.40</td>
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<tr>
<td>Maciasar R²⁶</td>
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<td>Type 1 and 2</td>
<td>PETIA</td>
<td>53</td>
<td>20</td>
<td>1</td>
<td>177</td>
<td>99mTc-DTPA</td>
<td>1.10</td>
</tr>
</tbody>
</table>
Forest plots of combined analyses are shown in figures 2-7. The sensitivity ranged from 0.81 to 0.98 (pooled sensitivity 0.88 95% CI 0.85, 0.91), while the specificity ranged from 0.67 to 1.00 (pooled specificity 0.85 95% CI 0.82, 0.87). The pooled positive likelihood ratio was 7.04 (95% CI 4.33, 11.43), while the negative likelihood ratio was 0.13 (95% CI 0.09, 0.20). DOR was 66.80 (95% CI 27.92, 159.86).
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Fig. 4. Forest plots of pooled positive likelihood ratio of serum cystatin C for the estimation of glomerular filtration rate in predicting diabetic nephropathy. The red circles and horizontal lines represent the study-specific index of diagnosis and corresponding 95% CI, respectively. The diamond represents the pooled estimate with 95% CI.

Fig. 5. Forest plots of pooled negative likelihood ratio of serum cystatin C for the estimation of glomerular filtration rate in predicting diabetic nephropathy in diabetes disease. The red circles and horizontal lines represent the study-specific index of diagnosis and corresponding 95% CI, respectively. The diamond represents the pooled estimate with 95% CI.

Fig. 6. Forest plots of pooled Diagnostic OR of serum cystatin C for the estimation of glomerular filtration rate in predicting diabetic nephropathy. The red circles and horizontal lines represent the study-specific index of diagnosis and corresponding 95% CI, respectively. The diamond represents the pooled estimate with 95% CI.

SROC analysis
SROC for 9 studies, AUROC = 0.9549, Q-test* = 0.8972, SE (Q*) = 0.0224 figure 7.

Discussion
Serum cystatin C is a non-glycosylated, low molecular weight and basic protein that is produced at a steady rate and can be completely removed from the circulation by glomerular filtration, with subsequent proximal tubular reabsorption and degradation [27, 28]. Several studies have demonstrated that the levels of serum cystatin C are not affected by age,
gender, and body mass and that its levels are not altered by inflammation conditions [28, 29]. The progression of DN is accompanied by a decrease in GFR among patients with DM. As the patient’s GFR falls below 60 ml/min/1.73 m\(^2\), there is an increased risk of death and cardiovascular mortality [30]. The present meta-analysis aimed to compare serum cystatin C with mGFR to predict DN.

The present meta-analysis enrolled 9 studies referring to the serum cystatin C test for the early prediction of DN. The results showed that the pooled sensitivity and specificity for detecting DN with the serum cystatin C were 0.88 and 0.85, respectively. The higher the positive likelihood ratio was, the greater the value of the diagnosis of disease was, whereas the smaller the negative likelihood ratio was, the lower the possibility was of suffering from the specific disease. Hence, it may be more appropriate to consider the likelihood ratio when explaining the results. The pooled positive likelihood ratio was 7.04, which showed that DM patients with elevated serum cystatin C were more likely to progress to DN. The pooled negative likelihood ratio was 0.13, which showed that patients with normal serum cystatin C had fewer incidents of DN. Additionally, the SROC curve was an overall evaluation of the value of the diagnostic tests. The present meta-analysis showed that the SROC area under the curve was 0.9549 and that the Q value was 0.8972. DOR is the ratio of the true positives to false positives compared to the ratio of the true negatives to false negatives. The diagnostic odds ratio was 66.80. Therefore, cystatin C is a more reliable predictor of DN among patients with DM.

The source of heterogeneity could be explained by the following limitations. There were three methods of measuring GFR. First, although most of the methods for measuring GFR in the studies used the \( ^{51} \)Cr-EDTA plasma clearance, the observation time was different. Second, most studies did not give the diagnostic criteria of diabetes.

Our analysis has several limitations. For instance, we did not consider the effects of different methods for detecting the concentration of cystatin C. Several collaborative works have shown comparative performances between the nephelometric and turbidimetric immunoassay methods [31-33] to eGFR, but the results are different. Leeflang et al. suggested that we should consider the paired nature of the estimates and their dependence on the threshold [34]. In this article, many studies did not report the diagnostic value of serum
cystatin C [18, 21, 22, 24], but the difference of the analysis of diagnostic threshold was not significant (p = 0.4796). Clinical settings and study populations that were different, such as by racial or ethnic group, may explain the cut-off value used in these studies. The subjects represented seven countries. Hence, it may be necessary for each center to have different serum cystatin C cut-off values for each clinical setting.

Recently, Amini S demonstrated that among Kurdish patients, DN patients with a high frequency of CC genotype of solute carrier family 2 facilitated glucose transporter member 1 (SLC2A1) T20882C (HaeIII) polymorphism compared with diabetic patients without DN. This study also revealed that the C allele of SLC2A1 HaeIII polymorphism was associated with higher cystatin C and eGFR, but there was no relationship between HaeIII polymorphism and glycosylated hemoglobin (HbA1c) or between fasting blood glucose (FBG) and microalbuminuria [35]. Vaidya VS et al showed that tubular dysfunction is an important component of the early course of DN [36]. Arun O showed that serum cystatin C values may indicate acute kidney injury (AKI) signs earlier than blood urea nitrogen and serum creatinine in DM patients undergoing Coronary Artery Bypass Graft Surgery [37]. The study by Ozturk SA et al. indicated that the lower cystatin C levels in Wistar Albino rats that were given pneumoperitoneum and theophylline are suggestive of lower renal injury [38].

**Conclusion**

In conclusion, the current article shows that serum cystatin C is a significant predictor of DN among patients with DM and that the cystatin C test is more economical and more convenient than the standard method for GFR. With the development of medical treatment level, DN remains a therapeutic challenge with major diabetic complications [39]. The early detection of DN is very important and can improve patient outcomes.

**Disclosure Statement**

The authors of this manuscript state that they do not have any conflict of interests and nothing to disclose.

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